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Terborg, Lydia

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ORIGINAL ARTICLE

Porous polymer monolithic columns with gold nanoparticles as an intermediate ligand for the separation of proteins in reverse phase-ion exchange mixed mode

Lydia Terborg [a], Jorge C. Masini [b], Michelle Lin [c], Katriina Lipponen [d], Marja-Liisa Riekolla [d], Frantisek Svec [a,*]

[a] The Molecular Foundry, E.O. Lawrence Berkeley National Laboratory, Berkeley, CA 94720, United States
[b] Institute of Chemistry, Department of Fundamental Chemistry, University of São Paulo, C.P. 26077, 05513-970 São Paulo, Brazil
[c] Department of Chemistry, University of California, Berkeley, CA 94720, United States
[d] Laboratory of Analytical Chemistry, Department of Chemistry, P.O. Box 55, FIN-00014, University of Helsinki, Helsinki, Finland

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ABSTRACT

A new approach has been developed for the preparation of mixed-mode stationary phases to separate proteins. The pore surface of monolithic poly(glycidyl methacrylate-co-ethylene dimethacrylate) capillary columns was functionalized with thiols and coated with gold nanoparticles. The "nal mixed mode surface chemistry was formed by attaching, in a single step, alkanethiols, mercaptoalkanoic acids, and their mixtures on the free surface of attached gold nanoparticles. Use of these mixtures allowed "ne tuning of the hydrophobic/hydrophilic balance. The amount of attached gold nanoparticles according to thermal gravimetric analysis was 44.8 wt.%. This value together with results of frontal elution enabled calculation of surface coverage with the alkanethiol and mercaptoalkanoic acid ligands. Interestingly, alkanethiols coverage in a range of 4.46…4.51 molecules/nm^2 signi"cantly exceeded that of mercaptoalkanoic acids with 2.39…2.45 molecules/nm^2. The mixed mode character of these monolithic stationary phases was for the "rst time demonstrated in the separations of proteins that could be achieved in the same column using gradient elution conditions typical of reverse phase (using gradient of acetonitrile in water) and ion exchange chromatographic modes (applying gradient of salt in water), respectively.

* Corresponding author. Tel./fax: +1 510 486 7964.
E-mail address: fsvec@lbl.gov (F. Svec).
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Introduction

Mixed-mode chromatography refers to chromatographic methods that utilize more than one type of interaction between the stationary phase and analytes in order to achieve their separation [1,2]. While early chromatographic methods preferred stationary phases with a strictly singular functionality,